Trimodal CAR⁺TCR⁺hnCD16⁺ iPSC-derived T Cells Co-Targeting Surface and Intracellular/Neoantigens Demonstrate Additive Effect on Overcoming Tumor Heterogeneity and Cancer Escape

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ABSTRACT

Background

Antigen escape and tumor heterogeneity remain significant hurdles to the development of curative treatments in many cancers. To address tumor heterogeneity, the introduction of pairs of chimeric antigen receptors (CARs) in donor T cells has been demonstrated, however, this adds complexity to a manufacturing process already challenged by cellular product consistency. To tackle tumor heterogeneity while maintaining product purity, we applied our induced pluripotent stem cell (iPSC)-derived T cell (iT) platform to design an off-the-shelf cell therapy capable of targeting multiple tumor antigens through complementary activation pathways, including targeting of both cell-surface antigens as well as intracellular/neoantigens.

Methods

A CAR construct targeting BCMA or MICA/B, a T-cell receptor (TCR)αβ targeting NYESO1 (1G4) or tumor-associated metabolite presented by MR1 (MC.7.G5), and a high-affinity non-cleavable (hnCD16) to promote antibody-dependent cellular cytotoxicity (ADCC) were engineered into iPSCs for use as a renewable starting source in deriving uniformly-engineered T cells. Resulting multiplexed-engineered CAR⁺TCR⁺hnCD16⁺ (trimodal) iPSCs were differentiated into T cells and the function of each individual edit was evaluated by preclinical models designed to represent tumor heterogeneity. In addition to in vitro mix-culture assays, a mixed cell disseminated in vivo model was used to mimic cancer heterogeneity and to evaluate the in vivo potency of trimodal iT cells at mitigating tumor heterogeneity and antigen escape.

Results

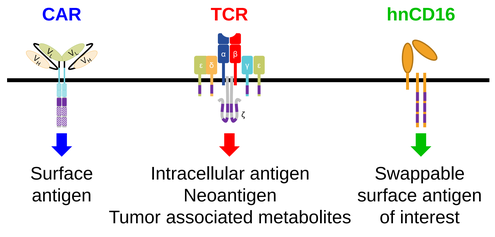
Assessment of individual edits in trimodal iT cells demonstrated independent functionality by exhibiting increased antigen-mediated IFNγ and TNFα production, and degranulation compared to the control group ($p<0.0001$). Using 9-day daily restimulation assay, each edit produced significant tumor reduction compared to tumor only control ($p<0.0001$). By stimulating trimodal iT cells with multiple antigens simultaneously using various solid tumor lines (A549, Caski and MDA-MB-231), we found that co-activation by two or three targeting edits significantly enhanced tumor killing ($p<0.0001$). Furthermore, when challenged with in vivo heterogenous tumor models, we found that the co-activation of all three targeting moieties in trimodal iT cells achieved nearly complete tumor clearance ($p<0.0001$). Ex vivo bone marrow analysis further confirmed antigen-specific target elimination, reenforcing the specificity and potency of the trimodal iT cells.

Conclusions

Our data highlight the potency and broad applicability of trimodal iT cell expressing CAR, TCR, and hnCD16. This consistent and scalable approach to multiplex-engineered T-cell therapy is an ideal strategy to mitigate antigen escape and combat difficult to treat heterogeneous solid tumors.

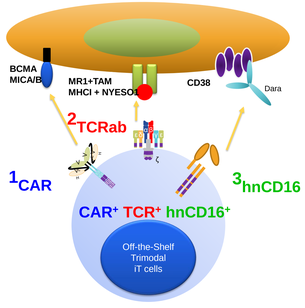
GRAPHICAL ABSTRACT

Enhance Specificity and Multi-antigen Targeting: Use of synthetic biology to integrate multiple antigen-targeting modalities into iPSC derived iT cells for enhanced anti-tumor activity



SUMMARY

- iPSC-derived Trimodal iT cells homogenously express three anti-tumor modalities, CAR, TCR, hnCD16, that are individually functional with specific activity.
- Trimodal iT cells demonstrate compatibility between CAR, TCR, hnCD16 synthetic receptors with additive anti-tumor efficacy.
- Trimodal iT cells represent an ideal strategy to create off-the-shelf cellular immunotherapy for a promising therapeutic approach to combat heterogeneous and difficult to treat solid tumors, including those that are resistant due to antigen escape.



RESULTS

iPSC Platform for Mass Production of Universal NK and T-Cell Products

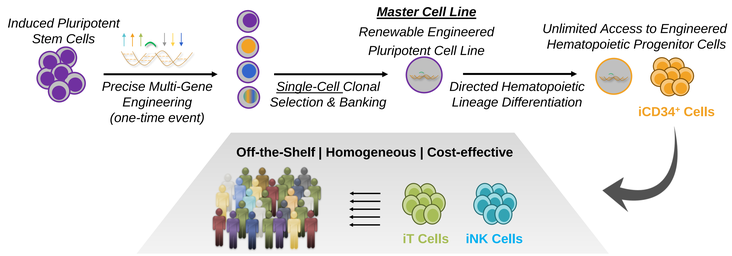


Figure 1. Fate Therapeutics induced pluripotent stem cell (iPSC) product platform enables mass production of off-the-shelf, engineered, homogeneous cell products that can be administered with multiple doses to deliver more effective pharmacologic activity, including in combination with cycles of other cancer treatments. Several programs are currently under clinical investigation.

Trimodal iT Cells Homogeneously Co-Express Three Individually Functional Anti-Tumor Modalities: CAR, TCR, and hnCD16

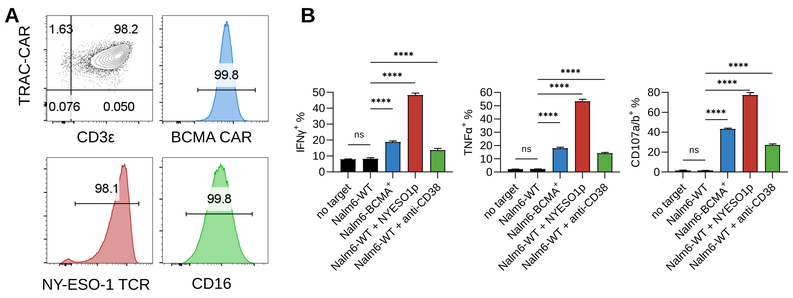


Figure 2. (A) FACS plots show the co-expression of TRAC-driven CAR and CD3ε to mark T cell identity and the expression of BCMA CAR, NYESO1 TCR, and hnCD16 on trimodal iT cells. (B) Graphs summarize the percentage of IFNγ, TNFα and CD107ab of CAR, TCR, or hnCD16 stimulated iT cells when stimulated with target cells engaging CAR only, TCR only, or hnCD16 only.

Trimodal iT Cells Demonstrate the Compatibility between CAR, TCR and hnCD16 to Enhance Anti-Tumor Efficacy

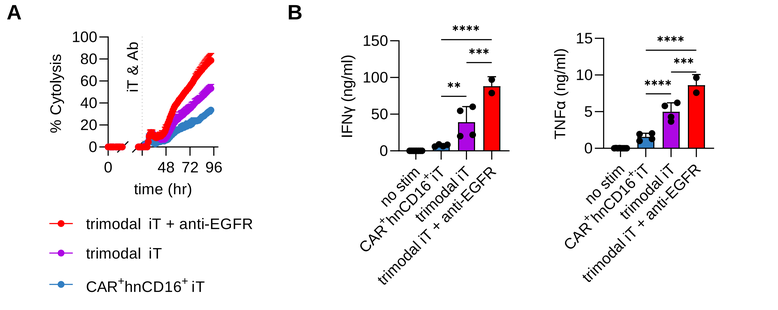


Figure 3. (A) Percentage of cytolysis of 3 independent replicates using A549 treated with indicated conditions. (B) IFNγ and TNFα production were evaluated 6 hours post tumor stimulation at 1:1 effector to tumor ratio and quantified by MSD assay.

Trimodal iT Cells (BCMA CAR⁺, NYESO TCR⁺, hnCD16⁺) Demonstrate Control of a Heterogenous Tumor Cell Population In Vivo Through Engagement of Three Antigen Targeting Strategies

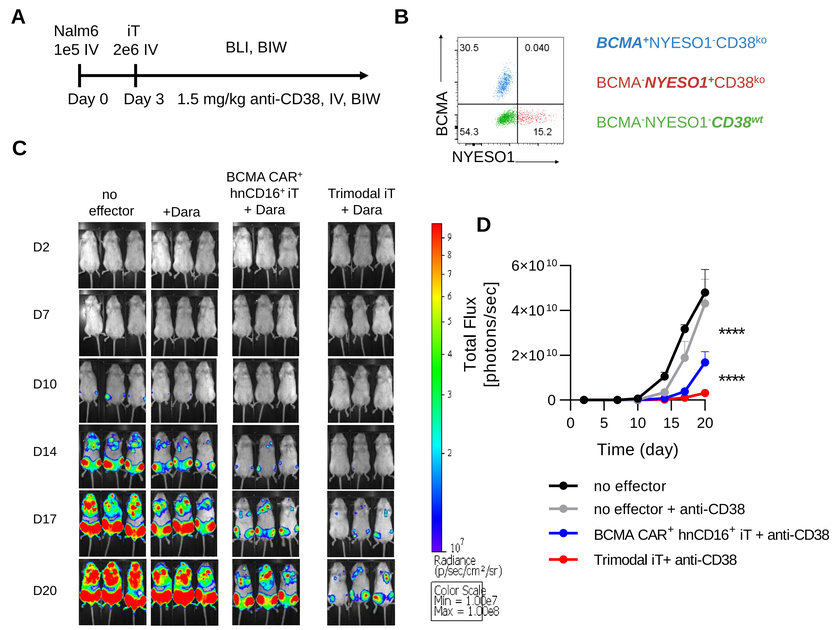


Figure 4. (A) Scheme illustrates Nalm6 model used in the study. (B) The FACS analysis of day 0 tumor input gated on CD10⁺ cells for BCMA-mCherry and NYESO1-GFP signals. (C) IVIS images demonstrate the tumor burden and location from D2 to D20. (D) Graph summary of BLI of indicated groups.

Ex vivo Bone Marrow Analysis from Xenograft Tumor Model Demonstrates Specific Targeting of Multiple Heterogenous Tumor Antigens by Trimodal iT Cells

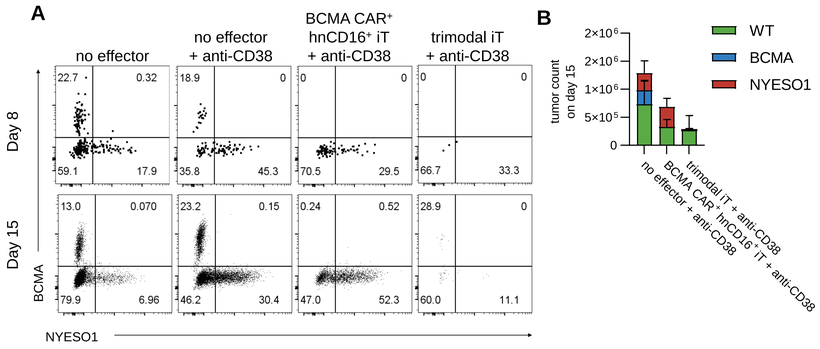


Figure 5. (A) The FACS analysis of *ex vivo* CD10⁺ tumor cells from bone marrow (BM) for BCMA-mCherry and NYESO1-GFP signals. (B) The absolute tumor count in each bone marrow sample for indicated tumor lines are plotted for all groups on experiment day 15.